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MICROBIAL ODOR PROFILE OF POLYESTER AND COTTON CLOTHES AFTER A FITNESS SESSION

Running title: Bacterial and odor profile of clothes

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ABSTRACT

Clothing textiles protect our human body against external factors. These textiles are not sterile and can harbor high bacterial counts as sweat and bacteria are transmitted from the skin. We investigated the microbial growth and odor development in cotton and synthetic clothing fabrics. T-shirts were collected from 26 healthy individuals after an intensive bicycle spinning session and incubated for 28h before analysis. A trained odor panel determined significant differences between polyester versus cotton fabrics for the hedonic value, the intensity and five qualitative odor characteristics. The polyester T-shirts smelled significantly less pleasant and more intense, as compared to the cotton T-shirts. A dissimilar bacterial growth was found in cotton versus synthetic clothing textiles. Micrococci were isolated in almost all synthetic shirts and were detected almost solely on synthetic shirts by means of DGGE fingerprinting. A selective enrichment of micrococci in an *in vitro* growth experiment confirmed the presence of these species on polyester. Staphylococci were abundant on both cotton and synthetic fabrics. Corynebacteria were not enriched on any textile type. This research found that the composition of clothing fibers promotes differential growth of textile microbes and, as such, determines possible malodor generation.

INTRODUCTION

Clothing textiles are in close contact with the microorganisms of the skin and those of the environment. The clothes create a warm and often moist environment on the skin, which leads to the growth of bacteria. In some cases, these microorganisms lead to unpleasant odors, staining, fabric deterioration and even physical irritation, like skin allergies and skin infections (1). The skin consists of various niches, each with its specific bacterial community present (2, 3). Very dry areas, such as the forearm, trunk and legs, harbor only 10^2 bacteria per cm^2, while the axillae, umbilicus and toe web spaces contain up to 10^7 bacteria per cm^2 (4). The human skin contains up to 19 different phyla (5) and even in one niche, the axillae, up to 9 different phyla are present (6). Skin microorganisms transfer to the clothing fibers and interact with these in several phases: adherence, growth and damage to the fibers. Growth of bacteria is due to sweat secretions, skin desquamation, natural particles present in the clothing fibers or on the fibers itself, or nutrition from elsewhere in the environment. An important factor determining bacteria-fiber interaction is the origin and the composition of the clothing textile. A large discrepancy exists in the way bacteria adhere to natural versus synthetic fibers. It is posed that natural fibers are more easily affected by the microbiota due to the natural nutrients present in the clothing and the ability to adsorb sweat components (1). Cellulose fibers are degraded by a range of bacteria and fungi, possessing cellulolytic enzymes (7). Synthetic fibers gather moister in the free space between the fibers but do not adsorb it on the fibers themselves. Synthetic fibers are therefore less susceptible towards bacterial breakdown, also due to the polyethylene terephthalate (PET) basis of the fiber (1).

Axillary malodor does not only emanate from the axillary skin but also from the textiles near the axillary region (8, 9). Dravnieks *et al*. (9) refers to this as the primary odor, originating from the axilla itself, and the secondary odor, originating from clothing in contact with the axilla. The odor would then differ between the two sites (10). It is found that a stronger body odor is generated by wearing synthetic clothing textiles as compared to natural textiles (10). This is held as a common belief; nevertheless, very few published data support this finding. Much research has nonetheless been conducted on controlling body odor by adding antimicrobials to textile fabrics (11-14).

Corynebacterium spp. are determined as the odor causing micro-organisms in the human

axilla (15). It is yet unclear which microorganisms are associated with the odor formation in clothing textiles. Few studies have been performed on determining the microbiota living in clothes. Therefore, this research focuses on (i) the determination of the microbial communities living in clothes, (ii) determining if different textiles host different communities, and (iii) determining the odor profile of different used fabrics after a sport session. This study focuses primarily on cotton (natural, consisting mainly of cellulose) versus polyester (synthetic) clothing textiles. An *in vivo* case study is performed on 26 healthy people, wearing 100% cotton, 100% polyester and intermediate cotton/synthetic clothing, doing a bicycle spinning session of one hour. A period of 28 hours is left between fitness and odor assessment, in order to let the bacteria grow on the textiles. A selected and trained odor panel assessed the odor of the individual T-shirts. The bacterial community is analyzed by means of denaturing gradient gel electrophoresis. An *in vitro* growth experiment is performed to analyze the selective enrichment of isolates on different clothing fabrics.

MATERIAL AND METHODS

Study design

First, an *in vivo* experiment was conducted with 26 healthy subjects, wearing cotton, synthetic and mixed cotton-synthetic T-shirts, participating in an intensive bicycle spinning session of one hour. The T-shirts were collected, sealed in plastic bags and stored at room temperature in the dark, so bacterial growth occurred. Axillary swabs were taken to analyze the bacterial community on the skin. Odor assessment by a trained odor panel and subsequent bacterial extraction was performed on the whole T-shirt. The individual samples were plated to obtain pure colonies for sequencing. The DNA was extracted from axillary and T-shirt samples and the microbial community was investigated by means of Denaturing Gradient Gel Electrophoresis (DGGE). Descriptive diversity and dynamics analysis was performed on the results. Second, an *in vitro* growth experiment was conducted in which typical skin/textile microbial isolates were incubated on a range of sterile textile fibers in order to identify the selective growth or inhibition on the textiles. Third, contact angle measurements were performed to detect the affinity of micrococci towards polyester and cotton textiles.

Sampling

Samples were taken from the T-shirt and the armpit skin of 26 healthy subjects (13 males and 13 females), participating in an intensive bicycle spinning session of one hour. The median age was 39 year (range 20y – 60y) (Table 1). Every subject wore a freshly washed T-shirt. All were in good health and had not received any antibiotics for at least two months. The participants had no history of dermatological disorders or other chronic medical disorders and had no current skin infections. No attempts were made to control the subjects diet or hygiene habits. All participants were residents living in the area of Willebroek (Belgium), with a temperate maritime climate by the North Sea and Atlantic Ocean. After one hour of intensive bicycle spinning, the T-shirts were aseptically collected and separately sealed in plastic bags. The bags were kept at room temperature (20°C) in the dark for 28 hours. This was done to simulate the home conditions and to let the microbial community grow on the specific clothing textiles. An axillary swab was taken from each participant, using a sterile cotton swab (Biolab, Belgium) that was formerly moistened with sterile physiological water. The swab was thoroughly swabbed for 15 s in the axillary region to detach and absorb the

121 microorganisms, after which the tip was broken in a sterilized reaction tube filled with 1.0 ml
122 of sterile physiological water (16). The bacterial samples were pelletized and frozen at -20°C
123 until DNA extraction.
124
125 ***Odor assessment***

126 Individual T-shirts in the plastic bags were presented to a panel of seven selected and
127 screened human assessors. Assessors were selected by means of sensitivity to dilutions of n-
128 butanol and wastewater, and by means of the triangle test (17). Each member of the panel was
129 presented three flasks, two of which were the same but the third contained a different odor.
130 The flask was shaken, the stopper was removed, after which the vapors were sniffed. The
131 panelists had to correctly identify the different flask. The triangle test was repeated three
132 times, with a minimum of two days in between each measurement. The room in which the
133 tests were conducted, was free from extraneous odor stimuli e.g. caused by smoking, eating,
134 soaps, perfume, etc. A representative team of odor assessors was chosen from the pool of
135 assessors. The odor assessors were familiar with the olfactometric procedures and met the
136 following conditions: (i) older than 16 years and willing to follow the instructions; (ii) no
137 smoking, eating, drinking (except water) or using chewing gum or sweets 30 min before
138 olfactometric measurement; (iii) free from colds, allergies or other infections; (iv) no
139 interference by perfumes, deodorants, body lotions, cosmetics or personal body odor; (v) no
140 communication during odor assessment. The samples were assessed by seven odor
141 characteristics: hedonic value (between -4 and +4), intensity (scale 0 to 6), musty (scale 0 to
142 10), ammonia (scale 0 to 10), strongness (scale 0 to 10), sweatiness (scale 0 to 10), sourness
143 (scale 0 to 10). A blank odor measurement, a clean cotton T-shirt with random number, was
144 served to the odor panel together with the other samples.
145
146 ***Statistical analysis odor characteristics***

147 The generated dataset from the odor assessment was statistically analyzed and visualized in R
148 (18). A heatmap and scatterplot were generated to visually interpret the correlations between
149 sensory variables. Significance cut-off values were set at 95% (α=0.05), unless otherwise
150 mentioned in the manuscript. Both a multivariate comparison of means as well as univariate
151 analysis were run after assessment of the hypothesis. Univariate normality was assessed using
152 a Shapiro-Wilks normality test. If normality could not be assumed, the Mann-Whitney (or
153 Wilcoxon rank sum) test was executed to assess null hypothesis of a location shift μ=0. The

alternative hypotheses were selected based upon exploratory data analysis. Non-available observations were handled by case-wise deletions. Multivariate datasets were analyzed on their normal distribution using Mahalanobis distances in Quantile-Quantile (QQ) plots. Also the E-statistic test of multivariate normality was executed (19). Multivariate homogeneity of group dispersions (variances) was assessed using the betadisper function from the package Vegan (20), an implementation of the PERMDISP2 procedure (21). Euclidean distance measures were used as well as the spatial median for the group centroid. The Hotelling's T^2 test was used to compare the multivariate datasets, comparing the multivariate means of each population (22). When necessary a Chi-squared approximation was used for the test to allow for relaxation of the normality assumption.

Bacterial extraction from T-shirts

The bacterial extraction occurred on the complete T-shirt, using TNE buffer (10 mM Tris–HCl pH 8.0, 10 mM NaCl, 10 mM EDTA) (23). 300 ml of TNE buffer was added to the plastic bag with the T-shirt, firmly sealed with tape and vortexed for 10 min. The buffer was subsequently manually pressed out of the T-shirt and transferred into sterile 50 ml reaction tubes. The extracts were respectively used for isolation of bacteria and for DNA extraction. The bacterial extraction procedure was chosen after an optimization procedure (Figure S1). The method focused on the extraction of the bacteria of the whole T-shirt. It was not possible to extract the bacteria from one region (e.g. axillary region) of the T-shirt. A clean T-shirt was extracted together with the other samples, as blank measurement.

Sanger sequencing of bacterial isolates

The microorganisms were isolated from the T-shirts by the standard method of dilution plating on nutrient agar. Incubation of all plates was performed at 37°C in aerobic conditions and facultative anaerobic conditions using a gas-pack cultivation jar. The colonies were plated three times on new agar plates using the streak plate method to obtain bacterial isolates. A total of 91 isolates was obtained. The isolates were transferred into a 1.5mL eppendorf with 50µL of sterile PCR water, vortexed and stored at -20°C to extract DNA. Dereplication was done using DGGE after amplification by PCR using 338F and 518R (24, 25). The analysis involved 31 nucleotide sequences. The 16S rRNA genes were subsequently amplified by PCR using 63F and 1378R (26). The PCR program were performed and checked as described below. Sanger sequencing was performed on the 16S rRNA amplicons, aligned and compared

187 with sequences from the NCBI database. The closest match of each isolate was identified.
188 Sequences of all strains were submitted to GenBank with the accession numbers KJ016241-
189 KJ016271. The bacterial isolates were constructed in an evolutionary taxonomic circular tree
190 (Figure 2) using the Neighbor-Joining method (27), conducted in MEGA5 (28). The tree has
191 branch lengths in the same units as those of the evolutionary distances used to infer the
192 phylogenetic tree. The evolutionary distances were computed using the Jukes-Cantor method
193 (29) and are in the units of the number of base substitutions per site. Codon positions included
194 were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence
195 pair. There were a total of 1172 positions in the final dataset.
196
197 ***DNA extraction, PCR & DGGE***

198 The bacterial solution in the TNE buffer was centrifuged for 5 min at 6000 *g*. The supernatant
199 was discarded and the obtained pellet was used for further DNA extraction. Total DNA
200 extraction was performed using the UltraClean Water DNA isolation kit (Mo Bio, USA). The
201 DNA was stored at -20°C until further analysis. The DNA extraction was chosen after a
202 comparative study of different DNA extraction methods (Figure S2). The 16S rRNA gene
203 regions were amplified by PCR using 338F and 518R (24, 25). A GCclamp of 40 bp (24, 25)
204 was added to the forward primer. The PCR program consisted of 10 min 95°C; 35 cycles of 1
205 min. 94°C, 1 min. of 53°C, 2 min. of 72°C; and a final elongation for 10 min. at 72°C.
206 Amplification products were analyzed by electrophoresis in 1.5% (wt/vol) agarose gels
207 stained with ethidium bromide. DGGE (Denaturing Gradient Gel Electrophoresis) was
208 performed as previously reported (6). A blank measurement was taken into account. To
209 process and compare the different gels, a homemade marker of different PCR fragments was
210 loaded on each gel (6). Normalization and analysis of DGGE gel patterns was done with the
211 BioNumerics software 5.10 (Applied Maths, Sint-Martens-Latem, Belgium). The different
212 lanes were defined, background was subtracted, differences in the intensity of the lanes were
213 compensated during normalization and bands and band classes were detected.
214
215 ***Selective growth of bacteria on textiles***
216 To analyze the selective growth of pure bacterial strains on different clothing textiles, bacteria
217 were inoculated and incubated on a sterile piece of textile in an *in vitro* growth experiment. A
218 wide range of clothing textiles was screened: polyester, acryl, nylon, fleece, viscose, cotton
219 and wool. Five common skin bacteria were grown on the textiles: *Staphylococcus epidermidis*

CC6 (GenBank KJ016246), *Micrococcus luteus* CC27 (GenBank KJ016267), *Enhydrobacter aerosaccus* (LMG 21877), *Corynebacterium jeikeium* (LMG 19049) and *Propionibacterium acnes* (LMG 16711). The bacteria were cultivated for 48h in nutrient broth, washed in M9 medium and finally dissolved in fresh M9 medium. A sterile piece of textile of 25 cm² was inoculated with 100 µl of the bacterial culture in a petridish. The inoculated bacteria were incubated for three days at 37°C. The bacteria were subsequently extracted using 10 ml of TNE buffer (23). The bacterial suspensions were measured using flow cytometry. To verify the extraction efficiency of the different clothing textiles, the bacterial strains were immediately extracted after inoculation using 10 ml of TNE buffer. All experiments were carried out in triplicate. A blank measurement, where bacteria were grown without textiles, was each time taken into account and deducted from the measurements.

Flow cytometry

Flow cytometry was used as a fast microbial measurement technique. The laser detection point of the device beams one cell at the time (λ_{max} = 488 nm), while the forward and side light scatter are detected. The samples were diluted 100 times in filtered Evian water (Danone Group, Paris, France) and stained with 1/100 SYBR® Green I dye (Invitrogen), as described in previous studies (30). The DNA-dye-complex absorbs blue light (λ_{max} = 497 nm) and emits green light (λ_{max} = 520 nm). Prior to flow cytometric analysis, the stained samples were incubated for 15 min in the dark at room temperature. Every sample was measured in triplicate, using the BD Accuri™ C6 flowcytometer (BD Biosciences, Belgium). The measurements were processed using the BD Accuri C6 software.

Contact angle measurements

The affinity of micrococci (*Micrococcus luteus* spp.) towards specific clothing textiles (cotton and polyester) was measured by means of contact angle measurements on the fabrics and the micrococci, as described earlier (31). Drops of three different solutes were applied on the tissues to determine Lifshitz-Van der Waals and electron-donor and –acceptor components of the surface tension, using the Young-Dupré equation and the extended DLVO approach (31). The solutes (milli-Q water, diiodomethane, and glycerol) had different physicochemical properties with known physicochemical parameters. As the textile fabrics absorbed much moisture due to the large voids between the fibers, contact angles were carried out on substitute materials: PET plastic to simulate polyester fibers, as PET is the basic substance for

polyester; and cardboard (cellulose) for cotton. *Micrococcus luteus* spp. were cultivated in nutrient broth for three days at 37°C. The bacteria were filtered on a 0.45 µm filter until a firm layer of micrococci was obtained, on which the contact angles were measured. Drop measurements were repeated at least 10 times for each liquid, whereby the average was taken. Anomalous measurements were rejected. All contact angles were measured using contact angle equipment (Krüss DSA10 goniometer, Krüss GmbH, Hamburg, Germany) equipped with contact angle calculation software (Drop Shape Analysis, Krüss GmbH).

Ethics Statement

The study was approved by the Ghent University Ethical Committee with approval number B670201112035. All participants gave their written consent to participate in this study as well as consent to publish these case details.

RESULTS

Odor differences between cotton and polyester clothing textiles

The hedonic value (the pleasantness of the odor) was qualified by the odor panel on a scale from -4 (very unpleasant) to +4 (very pleasant). The average hedonic value of 100% cotton T-shirts was -0.61 ± 1.08, while for 100% polyester T-shirts, a significantly lower value of -2.04 ± 0.90 was determined (Table S1). Polyester clothing after the spinning session smelled significantly less pleasant, and additionally, more intense, more musty, more ammonia, more strong, more sweaty and more sour (Figure 1). The qualitative differences were the largest for the sourness, strongness and mustiness. The dataset of the odor analysis was examined on its multivariate normal distribution by means of Mahalanobis QQ-plots (data not shown). Deviation from the bisector and, as such, from multivariate normality was observed, as confirmed formally by the E-statistic test ($p<0.05$). The multivariate means of cotton and polyester were compared to each other with the Hotelling's two sample T^2 test. This gave a p-value of 5.72×10^{-6}, meaning that a significant difference was found between the multivariate means of the cotton and polyester samples. The correlations between the different variables are visually represented in the heatmap in Figure S3. The t-test indicated no differences in deodorant/antiperspirant use among the 100% cotton and 100% polyester group ($p=0.86$) (Table 1).

Bacterial isolation and identification

Isolates of pure bacterial colonies were identified and are represented in Figure 2. A total of 91 isolates was obtained from aerobic and anaerobic plating. The isolates were screened by DGGE and sequenced to allow identification. Figure 2 represents 31 unique species found on the T-shirts. Not only Gram-positive but also many Gram-negative bacteria were found. Many skin-resident staphylococci were isolated from the textiles. Isolates also belonged to the genus of the Gram-positive *Bacillus* spp., Gram-positive *Micrococcus* spp., the Gram-negative *Acinetobacter* spp. and to the Gram-negative *Enterobacteriaceae* family, amongst others, which are generally not found on the axillary skin. The isolates were classified in three bacterial phyla: *Firmicutes*, *Actinobacteria* and *Proteobacteria*.

Bacterial fingerprinting of the textile microbiome

DGGE fingerprinting analyses showed large diversities amongst the individual shirts.

Although similar bacterial species were noticed, every textile microbiome was rather unique. Figure 3 shows the fingerprinting results of the 26 individual T-shirts. Apparent differences were found between cotton and synthetic clothing textiles after the fitness session. Particular bands were identified which correlated more with specific clothing fibers. *Micrococcus* spp. were predominantly found in synthetic clothing fabrics. Many micrococci were found on 100% polyester clothes, but also on mixed synthetic textiles, such as 82% polyester + 18% elastane. Micrococci were also found on mixed synthetic/natural textiles, such as 95% cotton + 5% elastane and 35% polyester + 34% cotton + 28% lyocell + 3% elastane (Figure 3). *Staphylococcus hominis* spp. bands were solely present on the 100% cotton clothing. *Staphylococcus* spp. were detected in relatively high amounts in practically all T-shirts. Individual DGGE fingerprinting was performed on both textiles and axillary skin (Figure S4). The axillary region was chosen as representative skin area, and compared with the textile microbiome, as both are known to generate malodor. Large differences were seen in the bacterial fingerprint patterns between the axillary and textile microbiome. An enrichment of skin bacteria on the textile was frequently observed, such as the apparent enrichment of *Staphylococcus epidermidis* spp. (Figure 3). The fingerprint results show that selective bacterial growth occurs in synthetic and cotton clothing.

Selective bacterial growth on clothing textiles

The selective growth of pure bacterial cultures was examined by means of an *in vitro* growth experiment on a range of different fabrics. The results, presented in Table 2, clearly indicated selective growth and inhibition for several species on the different fabrics. *Enhydrobacter aerosaccus* spp. and *Propionibacterium acnes* spp. were able to grow on almost every textile. Under the same conditions, *Corynebacterium jeikeium* spp. were not able to grow on the textiles, as the log counts decreased. *Staphylococcus epidermidis* spp. were able to grow on almost every textile, except viscose and fleece. *Propionibacterium acnes* spp. showed a remarkable growth on nylon textile, with bacterial counts up to 2.25×10^8 colony forming units (CFU) per cm². The log count difference among textiles was the most dissimilar for *Micrococcus luteus* spp. The largest growth was noted on polyester textiles (one log growth increase; up to 1.72×10^7 CFU per cm²), whereas the largest inhibition was noted on fleece textiles. This experiment confirmed the finding of selective growth of *Micrococcus* spp. on polyester clothing textiles, as well as no selective growth of *Micrococcus* spp. on cotton textiles. According to these results, viscose did not permit any growth of bacterial species. Wool, on the other hand, supported the growth of almost all bacteria. Nylon showed very

334 selective bacterial growth. The growth of *Staphylococcus*, *Propionibacterium* and
335 *Enhydrobacter* spp. was enhanced, while the growth of *Micrococcus* and *Corynebacterium*
336 spp. was inhibited. Growth on fleece likewise showed a selective profile. *Enhydrobacter* spp.
337 were enhanced; *Propionibacterium* and *Corynebacterium* spp. remained at the same level;
338 while *Staphylococcus* and *Micrococcus* spp. were inhibited. No growth (or inhibition) was
339 observed on acryl textile for practically all species. Cotton textile indicated a growth for
340 *Propionibacterium*, *Staphylococcus* and *Enhydrobacter* spp., while practically no growth (or
341 inhibition) was noted for *Micrococcus* and *Corynebacterium* spp. Polyester textile showed the
342 largest growth for *Propionibacterium*, *Enhydrobacter* and *Micrococcus* spp. Inhibition was
343 recorded for *Corynebacterium* spp. on polyester. No growth (or inhibition) was noted for
344 *Staphylococcus* spp.
345
346 **Contact angle measurements**
347 A potential explanation for the selective growth is a dissimilar non-electrostatic attraction
348 between the bacterium and the different textile surfaces. Contact angle measurements were
349 carried out (Table S2) to determine the attraction or repulsion for *Micrococcus luteus* spp.
350 towards cotton (cellulose) and polyester (PET). Using the Young-Dupré equation, the contact
351 angles were transformed into surface tension components, represented in Table S3. The
352 interaction energy between micrococci and cotton (ΔG = -1,22 ±1,00 J) was in the same range
353 as the interaction energy between micrococci and polyester (ΔG = 0,24 ±1,00 J). Both values
354 were determined around 0. No differences were found in interaction energy of micrococci -
355 cotton and micrococci - polyester.
356
357

DISCUSSION

It is generally accepted that the choice of clothing has an impact on malodor formation (10). This research showed that polyester clothes create a significantly higher malodor as compared to cotton clothing after a fitness session and an incubation period. Significant differences were found for the hedonic value and the intensity of the odor, as well as all qualitative odor characteristics (musty, ammonia, strongness, sweatiness and sourness). This corroborates earlier findings, where higher odor intensities were detected in polyester fabrics (10). A first reason for the different odor profile is explained by the difference in odor adsorbance. Polyester is a petroleum-based synthetic fiber and has no natural properties. Synthetic fibers hence have a very poor adsorbing capacity, due to their molecular structure. Cotton is a natural fiber, originating from the *Gossypium* cotton plants. These cotton fibers almost purely consist of cellulose, which has a high adsorbing capacity (32). Next to moisture, odors are adsorbed, and less malodor is emitted. A second reason can be explained by the dissimilar bacterial growth on the different textiles, where the malodor causing *Micrococcus* spp. tends to grow better on synthetic textiles. The poor adsorbing properties and the selective bacterial growth of micrococci may account for the malodor emission by certain synthetic sport clothes.

The microbial community of the textiles differs with the community living on the axillary skin (Figure S4). While the axillary microbiome is generally dominated by *Staphylococcus* and *Corynebacterium* species (6), the textile microbiome was rather dominated by *Staphylococcus* and *Micrococcus* spp. (Figure 3). The three main bacterial phyla found in the textiles (*Firmicutes*, *Actinobacteria* and *Proteobacteria*) are also three important phyla of the skin microbiome (5). Certain species were able to grow in more abundant quantities on the textile fibers. It is suggested that malodour generation is associated with the selective growth of those species. The bacterial enrichment was studied and differed depending on the bacterial species and the type of clothing textile, as shown by an *in vitro* growth experiment (Table 2). Micrococci were selectively enriched on polyester and wool, but were inhibited on fleece and viscose. Polyester textiles showed an enrichment for *Micrococcus*, *Enhydrobacter* and *Propionibacterium* spp. These enrichments can have an important impact on the malodor creation from excreted sweat compounds. *Staphylococcus epidermidis* spp. were enriched on both cotton and polyester textiles, as seen in the fitness clothes (Figure 3). These results are in

close correlation with previous findings, where a high affinity of *Staphylococcus* spp. for cotton and polyester was reported (33, 34). The enrichment was confirmed by the *in vitro* growth experiment, with a growth reaching up to 10^7 CFU per cm² textile for cotton, wool and nylon. On polyester, the presence was maintained on a level of 10^6 CFU per cm². Additionally, *Staphylococcus hominis* spp. were often able to gain dominance on cotton textiles, as seen in the fitness experiment. This was not seen for synthetic clothing textiles. No bacterial enrichment was seen on viscose, textile made from regenerated wood cellulose. Viscose showed very low bacterial extraction efficiencies. Further research is needed to confirm the absence of bacterial growth on viscose. If bacterial growth is indeed difficult to occur on these fiber types, viscose could be used as bacterial and odor preventing textile in functional clothes. Wool, on the other hand, promoted the growth of almost all bacteria. This is in correlation with earlier findings, where the highest bacterial growth was noted for wool, as compared to other tested clothing textiles. Although high bacterial counts, the odor intensity ratings were the lowest for wool (10). Nylon showed a very selective bacterial growth, with the biggest enrichment noted for *Propionibacterium* spp. (up to 10^8 CFU per cm²). *Staphylococcus* and *Enhydrobacter* spp. were enhanced as well, while the growth of *Micrococcus* and *Corynebacterium* spp. were inhibited. The *Propionibacterium* spp. are known to cause an acidic, intense foot odor (35). The enrichment of these species on nylon socks have an important consequence on the foot malodor generation.

The *Corynebacterium* genus was not able to grow under the circumstances of the *in vitro* growth experiment. The genus was likewise not detectable on DGGE or could not be isolated from any clothing textile after the fitness experiment, although it was initially present in the axillae of many subjects (Figure S4). These findings are consistent with previous findings, where no growth of corynebacteria on clothing textiles was found (10, 34). Corynebacteria are generally known as the most important species causing axillary malodor (36). These bacterial species are thought to be involved in the conversion of sweat compounds into volatile short branched-chain fatty acids, steroid derivatives and sulphanylalkanols, the three main axillary malodor classes (15). The results of this study, together with former research, indicated that corynebacteria are not the abundant bacterial species on clothing textiles. The absence or inability of corynebacteria to grow on clothing textiles implies that there are other bacterial types involved in the malodor creation in fabrics.

This research showed an overall enrichment of micrococci on the synthetic fabrics after the

fitness session and incubation period. The bands were clearly visible on DGGE, meaning that the bacteria were present for at least more than 1% of the bacterial community (37). Isolates of *Micrococcus* spp. were identified not only in 100% polyester textiles, but also in almost every shirt where synthetic fibers were present (Figure 3). The results were confirmed by the *in vitro* growth experiment (Table 2). Of the seven tested textile types, micrococci were able to gain the highest abundance on polyester fabrics (up to 10^7 CFU per cm²). No selective growth was found for micrococci on cotton textiles after three days. Previous research found one single enrichment of micrococci on polyester (34). These findings confirm that micrococci are selectively enriched on polyester fabrics. It is hypothesized that the circumstances on synthetic clothing textiles is favorable for the growth and activity of *Micrococcus* spp. Their enrichment was not caused by a higher non-electrostatic adsorption affinity for polyester. Other factors play a role in the enrichment of the micrococci. The aerobic growth conditions on polyester favor the growth of aerobic micrococci. Bacteria in clothing textiles are no longer suppressed by the innate immune system present on the skin. The nutritious environment, as well as quorum sensing (38, 39), can additionally play a role in the growth of micrococci. A multiplicity of these favorable situations causes the selective enrichment of micrococci on polyester fabrics. *Micrococcus* spp. are known for their ability to create malodor from sweat secretions. They are able to fully catabolize saturated, mono-unsaturated and methyl-branched fatty acids into malodor compounds (4, 40). Next to corynebacteria, micrococci have been held responsible for the formation of body odor. These species have a high GC% content and are related to corynebacteria (both are members of the *Actinobacteria* phylum). Micrococci were frequently found in the axillary region, yet always by means of culturing techniques (4, 41). In molecular studies, micrococci have not been found in large quantities on the human axillary skin (6, 42). We suggest that micrococci were detected as they preferentially grow on the textiles worn close to the axillae and due to the practice of culturing techniques, which favor the growth of micrococci. It is suggested that micrococci prefer the aerobic environment of the textile fibers, while corynebacteria prefer the lipid-rich and more anaerobic environment on/in the (axillary) skin (43). This may also explain the odor differences frequently perceived between axillary skin and the textile worn at the axillary skin. The use of underarm cosmetics may additionally impact the skin microbiome and the subjects body odor. Stopping or resuming deodorant/antiperspirant usage leads towards an altered underarm microbiome. Especially the use of antiperspirants causes significant changes (44). Other factors include the general hygiene habits (frequency of washing, soap/shower gel type, etc.), the occupational lifestyle (physical activities, food

habits, etc.) and the environment (place of residence and work, climate, humidity, etc.) which can impact the skin microbiome.

This research indicated that enrichment of micrococci occurred on polyester, and in general, on synthetic clothing textiles. They were frequently isolated, identified by means of DGGE fingerprinting and enriched by an *in vitro* growth experiment on these textiles. The odor of the synthetic textiles was perceived as remarkably less pleasant, after an intensive sport session. Microbial exchange occurs from skin to clothing textiles. A selective bacterial enrichment takes place, resulting in another microbiome as compared to the autochthonous skin microbiome. The enrichment depended on the type of clothing textile and the type of bacterial species. With the current knowledge, the textile industry can design adjusted clothing fabrics which promote a non-odor causing microbiome. This research opens perspectives towards better and functionalized sports clothing, which emit less malodor after use. Antimicrobial agents may be added to washing machine powders specifically against the odor causing microbiota, rather than using broad-spectrum antimicrobials. The enhancement of the non-odor causing bacteria and the inhibition of the odor causing bacteria, which are enriched on certain textiles, could greatly improve the quality of the fabrics.

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FIGURE LEGENDS

Figure 1: Odor characterization of cotton (green) and polyester (red) clothing after a fitness experiment, assessed by the odor panel. The hedonic value was assessed between a value -4 (very unpleasant), 0 (neutral) and +4 (very pleasant) and rescaled between 0 and 8. The intensity represents the quantity of the odor, in a value between 0 (no odor) and 10 (very strong/intolerable). The qualitative odor characteristics musty, ammonia, strongness, sweatiness and sourness were assessed between 0 and 10. The odor assessment is represented in boxplots, with the middle black line as median odor value and the little circles as the outliers. Polyester clothing smelled significantly more after a fitness session than cotton.

Figure 2: Bacterial isolates obtained from the T-shirts after the spinning session represented in an evolutionary taxonomic circular tree, using the Neighbor-Joining method.

Figure 3: DGGE bacterial profile of 26 individual T-shirts after the bicycle spinning session. The legend on the right represents the subject number and the textile fibers, with P = polyester, C = cotton, E = elastane, L = lyocell. The samples were separated between cotton and synthetic clothing fibers.

TABLE LEGENDS

Table 1: Metadata of the participating subjects.

Table 2: Growth or inhibition (in log numbers) of bacterial species after a three day inoculation on different clothing textiles. The average CFU/cm² of the triplicates were represented together with the standard deviation. A color code is given according to the log growth or reduction compared to the initial bacterial concentration.

642 # TABLES

643 Table 1

Subject	Gender	Age	# wash per week	# deo per week	Textile type
1	M	36	10	1	100% polyester
2	F	28	10	7	82% polyester + 18% elastan
3	M	29	12	7	100% cotton
4	M	52	7	7	100% cotton
6	M	40	7	7	100% polyester
8	M	44	9	7	100% polyester
9	M	36	7	10	100% polyester
10	F	43	7	7	100% cotton
11	M	42	7	9	100% polyester
12	M	32	7	0	100% polyester
13	F	35	7	0	100% polyester
14	F	42	7	0	100% cotton
15	F	41	7	10	34% cotton + 28% lyocel + 35% polyester + 3% elastan
16	F	60	7	14	95% cotton + 5% elastan
17	M	42	12	0	100% cotton
18	F	54	7	7	95% cotton + 5% elastan
19	M	21	7	10	100% cotton
20	M	56	7	7	100% cotton
21	F	30	7	9	95% cotton + 5% elastan
22	F	49	14	7	100% cotton
23	F	20	6	7	100% polyester
24	M	31	10	5	100% cotton
25	F	43	7	10	100% cotton
26	M	38	4	9	100% polyester
27	F	37	7	7	100% polyester
30	F	36	4	9	95% cotton + 5% elastan

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645 Table 2

	Initial bacterial conc.	Cotton	Acryl	Wool	Viscose	Nylon	Fleece	Polyester
Staphylococcus epidermidis	(6.94±0.53) x 10^6	(1.04±0.67) x 10^7	(3.00±0.86) x 10^6	(9.76±9.70) x 10^7	(9.09±7.00) x 10^4	(8.96±13.74) x 10^7	(8.05±2.70) x 10^5	(5.19±4.43) x 10^6
Propionibacterium acnes	(8.69±0.29) x 10^5	(2.34±4.04) x 10^7	(2.79±1.95) x 10^5	(3.58±3.16) x 10^7	(7.68±3.11) x 10^5	(2.25±1.76) x 10^8	(8.72±10.16) x 10^5	(2.65±0.52) x 10^7
Corynebacterium jeikeium	(3.88±1.16) x 10^6	(6.70±3.17) x 10^6	(1.34±0.86) x 10^6	(1.08±1.34) x 10^6	(5.05±1.75) x 10^4	(4.04±2.02) x 10^5	(1.34±1.23) x 10^6	(5.39±4.20) x 10^5

Micrococcus luteus	(8.45±0.05) x 10^6	(7.81±7.56) x 10^6	(1.40±0.40) x 10^6	(1.21±1.44) x 10^7	(8.42±3.25) x 10^4	(2.90±1.37) x 10^6	(2.53±1.69) x 10^5	(1.42±0.57) x 10^7
Enhydrobacter aerosaccus	(9.66±0.42) x 10^5	(4.53±0.78) x 10^6	(1.93±1.04) x 10^6	(2.30±2.33) x 10^7	(6.26±0.35) x 10^5	(2.44±1.23) x 10^7	(1.44±0.76) x 10^6	(2.30±0.21) x 10^7

Legend	-2 log	-1 log	0 log	+1 log	+2 log	+3 log

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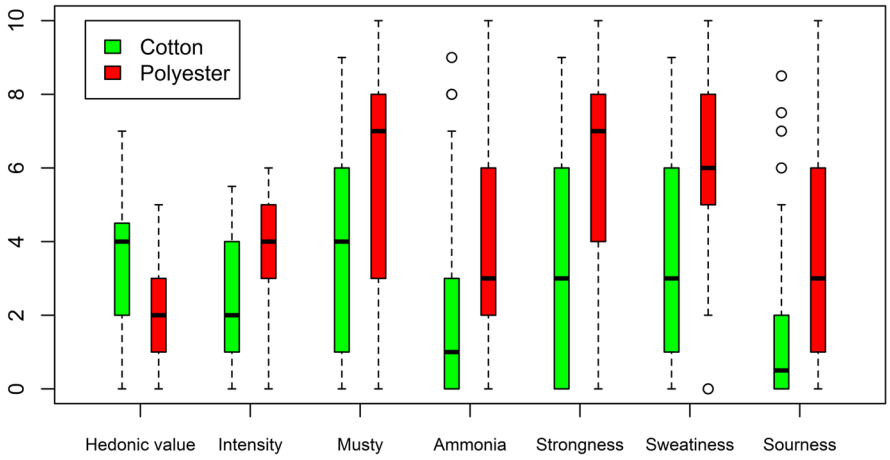

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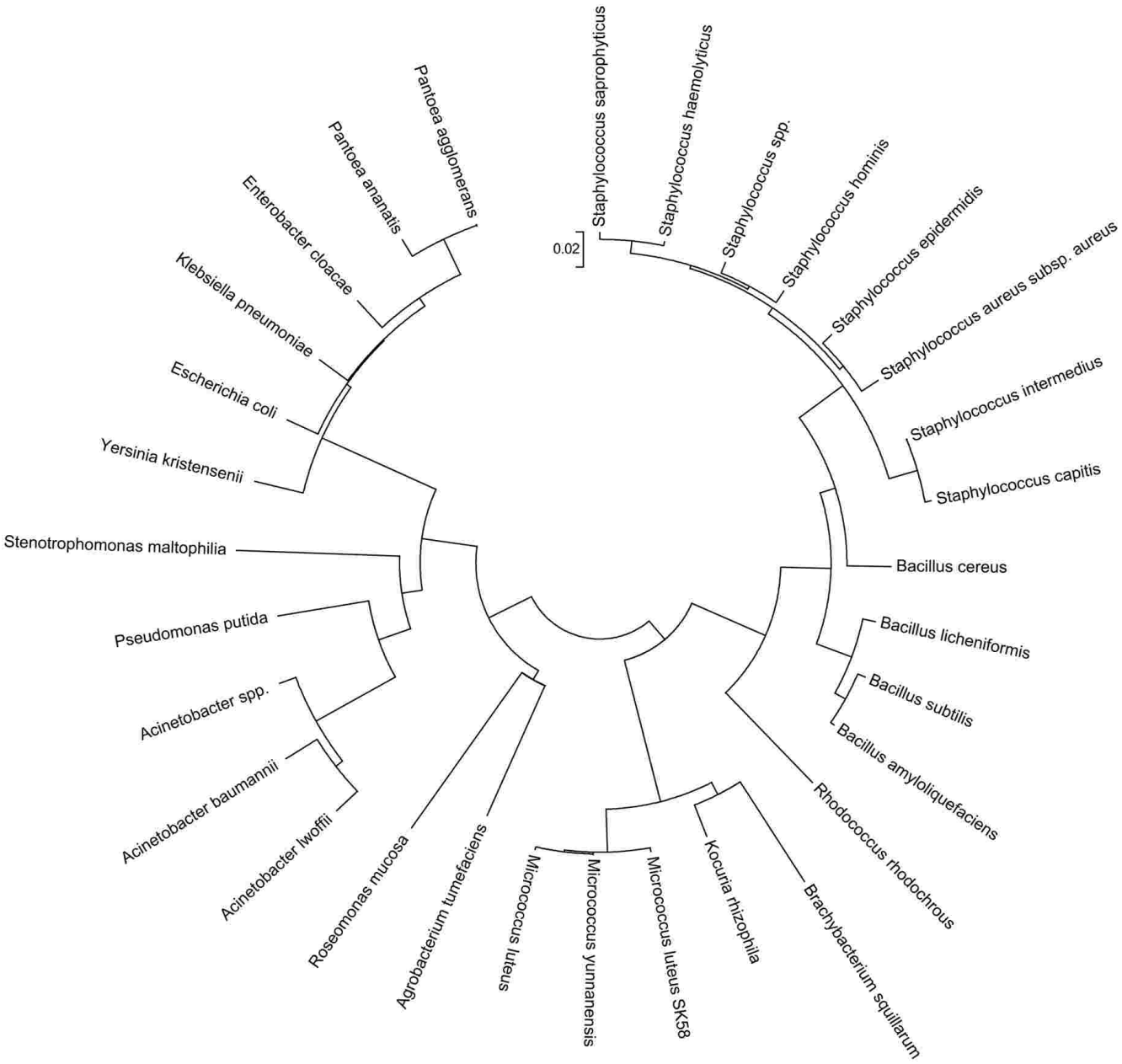

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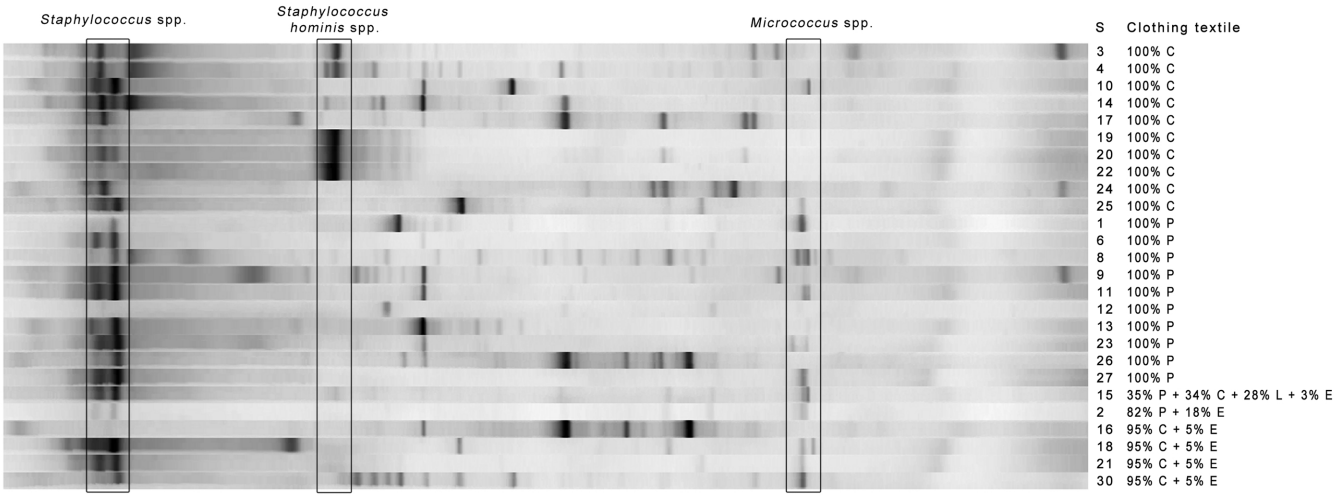

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